Genetic variation of *Cicer arietinum* among different varieties in the region of Marathwada, Maharashtra.

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Swami Ramanand Teerth Marathwada University, Nanded (Maharashtra)

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Abstract of proposed work plan/ problem

Genetic variation of *Cicer arietinum* among different varieties in the region of Marathwada, Maharashtra.

1. Introduction (Definition of problem):

Chickpea (Cicer arietinum L.) also known as Bengal gram or simply gram and as "chana" in Hindi and " Harbhara" in Marathi is a major rabi pulse crop. It is an autogamous annual herb belonging to sub family papilionaceae of the family leguminceae having chromosome number 2n = 16. It is believed to have originated in South-Western Asia and has been grown traditionally throughout the semi-arid regions of India and the Mediterranean

Chickpea is the third most important pulse crop of the world (after dry bean and pea) cultivated over an area of 11.15 million hectares with production and productivity of about 8.58 million tones and 769 kg/ha, respectively. The most important chickpea growing countries are India, Pakistan, Turkey, Iran, Mexico, Myanmar, Ethiopia, Australia and Canada. India, ranks first in the world sharing 65 and 67 per cent of the total global area and production, respectively. In India, chickpea is cultivated on an area of 7.1 million hectares with production of 5.6 million tones and productivity of 795 kg/ha. Major chickpea producing areas are concentrated in Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Haryana and Punjab. In Rajasthan, the crop is cultivated mainly in the districts of Churu, Jhunjhunu, Bikaner, Hanumangarh, Sikar, Jaipur, Sriganganagar, Tonk, Nagaur and Ajmer occupies 10.11 lack hectares area and produces 8.73 lace tones of grain with a productivity of 864 kg/ha.

Nutritionally, it is very rich as it contains about 17-21% protein, 62% carbohydrate and 5.3% fat. Chickpea is also rich in Ca, Fe, vitamin C (in green stage) and vitamin B1. Cereal and chickpea proteins are nutritionally complementary, the amino acids which are deficient in one being generally adequate in other. The P.E.R (protein efficiency ratio) value of chickpea seems to be the highest among grain legumes. It is used in many forms as „dal", „chhole" and „besan" (gram flour). Gram flour is used for the preparation of sweets, snacks and many attractive dishes. Its leaves contain mallic and citric acid, which are very useful for stomach ailments and blood purification. Its straw is highly rich in nutrients and mostly used as productive ration for animals.

Chickpea is adversely affected by several biotic stresses such as diseases viz., Fusarium wilt, gray mould and Ascochyta blight, and insect pests, mainly pod borer (Helicoverpa armigera) and abiotic stresses. Because of these factors the area under chickpea has declined considerably. Scope of increasing production lies in thorough understanding of chickpea ideotype and increasing biomass production, harvest index and insect resistance, resistance to drought, salinity and alkalinity, etc.

Chickpea is a self-pollinated crop and the scope for exploitation of hybrid vigor will depend on the direction and magnitude of heterosis and type of gene action involved. The study of heterosis and inbreeding depression will have a direct bearing on the breeding methodology to be employed for varietal improvement.

Different type of problems concern with crop production. geographical conditions play important role in crop yield, that's why each district of Marathwada, Maharashtra differ in crop yield. Also, genetic

makeup of chick pea an important factor considers in maximum crop yield. So, assessment of morphology and molecular data of chick pea some biometric techniques play essential role in defining problem.

RAPD, RFLP, AFLP and microarray are some methods useful in explaining genetic variation of different varieties of chick pea and their effect on crop yield. All these techniques analyze genetic variation of chick pea and based on these molecular data; researcher able to explain how exactly molecular approach of chick pea impact on crop yield.

With this background the present investigation will focus to estimate the relative magnitude of different types of gene effects and their interactions for yield and its components in chickpea among different region of marathwada, Maharashtra. What different varieties of chick pea in different region impact on crop yield is under studying.

2. Review of Literature and development in subject (Previous work done in the relevant area):

Chickpea (*Cicer arietinum* L) is a major pulse crop in the world. The harsh environmental conditions under which chickpeas are generally grown impose restrictions on the expression of genetic yield potential. As it is a very important pulse crop in India, the efforts were and are being made by the scientists from time to time to improve its productivity. The information's available on the genetics of yield and yield contributing attributes in chickpea, which is of fundamental importance in devising breeding methods, are very scanty and particularly very limited under varied environment. The available literature on the genetics of yield and yield attributes in chickpea is reviewed in this chapter along with a general review on the methods used in quantitative genetics to obtain information on the above aspects.

Plant breeders are primarily interested in improving yield by developing superior varieties. This can be achieved by altering the genetic makeup of existing varieties. The phenotypic expression of yield and most of the other characters of economic importance is quantitative in nature and their inheritance not only involves large number of genes with small individual effects, but these genes also interact themselves in a complex manner and are highly influenced by environment. According to Smith (1944) typical quantitative characters were determined by a large number of genes, sometimes referred to as polygene's (Mather, 1943) having small individual effects and acting in cumulative manner. Two classical experiments of Scandinavian geneticists (Johannsen, 1909 and Nilsson-Ehle, 1909) explained that similar factors of smaller individual action could account for continuous variation. Each of these factors would follow the similar mendelian inheritance pattern. Fisher (1918) carried the integration of biometry and genetics and partitioned the genotypic variance into three logical components namely,

(i) average effects of genes (additive genetic variance)

(ii) allelic interactions of segregating genes (dominance variance) and

(iii) non-allelic interactions of segregating genes (epistatic variance).

Wright (1935) termed these as

(i) additive genetic variance

(ii) variance due to dominance deviation and

(iii) variance due to epistatic deviation, respectively.

Fisher et al. (1932) presented a method for estimation of degree of dominance. Mather (1949) partitioned genetic variance into fixable (D) and non-fixable (H) components assuming no epistasis in F2, F3 and back cross generations derived from two homozygous parents. Several other eminent biometricians like Wright (1921), Haldane (1932), Cockerham (1954), Kempthorne (1957), Rawling and Cockerham

(1962), Gardner (1963), Dudley and Moll (1969) and Mather and Jinks (1971) have made commendable contribution in the partitioning of total genetic variance. Further, the epistatic variance was partitioned into factorial components of digenic and higher order interactions viz., additive x additive, additive x dominance and dominance x dominance for two loci by Cockerham (1954).

Mather (1949) was first to envisage a three-parameter model and described the effect of gene differences on the phenotypic expression by two parameters i.e., additive (d) and dominance (h) with „m" being mid-parent value. Considering epistasis not important, he suggested transformation of scale to fit the data of this non-epistatic model. The scale appropriate for one population may not be appropriate for another and scale appropriate to genetic and environmental components of variation may be different (Powers, 1951). Further, Mather (1949) and Hayman and Mather (1955) proposed „A", „B", „C" and „D" scales in order to determine the presence or absence of gene interactions.

Cavalli (1952) proposed a joint scaling test for estimating parameters, m, (d), and (h) with the help of weighted least square technique which was based on four or more number of families and to test the adequacy of the additive-dominance model by chi-square technique. A detailed treatment of relationship between scale and epistatic interaction is given by Horner et al. (1955). They concluded that a transformation, which reduced interaction variance, might be useful, if conclusions were to be drawn from an analysis that depends on its validity in the absence of interaction.

Analysis of genetic variance applying second degree statistical method into components like additive and non-additive and of the later into dominance and epistatic variances lead to devising appropriate selection methods as well as the breeding methods in a crop. A number of methods have developed which enable a breeder to obtain estimate of the components of the genetic variance.

The frequently adopted ones are:

(1) Co-variance of half sib and full sibs (Comstock and Robinson, 1948 and 1952)

(2) Diallel cross analysis (Hayman, 1954)

(3) Line x tester analysis (Kempthorne, 1957)

(4) Combining ability analysis (Sprague and Tatum, 1942, Griffing, 1956 etc) These methods were developed on the principles given by Fisher (1918 and 1932).

Pandey and Tiwari (1989) estimated the nature and magnitude of digenic allelic interactions involved in the expression of yield and its components by generation means under two environments. The results showed that plant height, primary branches and secondary branches inherited by simple additive and dominance gene effects in all the crosses. While, both additive and non-additive gene effects (dominance and epistasis) were important for flowering and maturity in majority of the crosses.

Shinde and Deshmukh (1990) carried out scaling test with five generation means in five crosses of chickpea to elucidate the type of gene action involved in the expression of yield and its contributing characters. The results indicated that epistatic gene actions were involved in the expression of fruiting branches per plant, pods per plant, 100-grain weight, yield per plant and days to maturity. In all the five crosses dominance gene action was involved for all the characters i.e., fruiting branches per plant, pods per plant, yield per plant and days to maturity except for 100-grain weight. In most of the crosses, additive gene action was involved in the inheritance of 100-seed weight. But additive and dominance gene effects, dominance x dominance and additive x additive interactions were important for all the yield components. Dominance effects followed by interactions and additive component played a significant role in the inheritance of grain yield. Duplicate epistasis was more predominant.

Kumar et al. (1999) carried out genetic analysis for different components of crop duration in 6 accession and 15 F1 hybrids of chickpea. The results indicated that additive and non-additive gene actions were involved in the inheritance of days to first flower, days to first pod, days to maturity, total reproductive period, pod establishment period and pod filling period with additive gene effect being predominant in the expression of the days to first flower, days to first pod, days to maturity and non-additive gene effects for the total reproductive period, pod establishment period and pod filling period.

Gupta and Ramanujam (1974) observed heterosis in chickpea for grain yield and reported 122 percent higher grain yield in hybrid than the better parent.

Gowda and Bahl (1976) concluded that heterosis for yield was the result of heterosis for pods per plant and number of branches per plant. For seed size, majority of the crosses showed negative heterosis.

Singh (1976) revealed that the heterosis in yield was mainly due to heterosis in pod number. For seed size none of the crosses gave higher value in comparison to high parent.

3. Objective of Research / Proposed Hypothesis:

A] Analysis of morphological features and yield of different varieties of chick pea in region of Marathwada,

B] To obtain the molecular data of chickpea accessions and wild species analysed by RAPD markers

C] To obtain the molecular data of chickpea accessions and wild species analysed by RFLP approach

D] To estimate the phylogenetical and evolutionary relationship among the selected species of chickpea accessions and wild species using DNA barcoding.

E] Assessing impact of genetic diversity on crop yield

4. Methodology to be adapted:

A. Study area:

The present study will be carried out at eight districts of Marathwada region of Maharashtra viz as Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani. Study will be proceeding on different verities of sample of chick pea from each district of Marathwada region of Maharashtra.

B. Collection of samples:

Sample of chick pea is collected from whole Marathwada region of Maharashtra. Sample of 4-5 different verities of chick pea is collected from each district of Marathwada region of Maharashtra. 100 gm of each sample of chick pea used for further investigation.

or

Plants: Seeds of the chickpea accessions and wild species will be obtained from ICAR, Pusa, New Delhi, and various Agriculture universities of different localities. Plants will be grown in pots in a glasshouse. The cultivated accessions and wild species will be chosen on the basis of their importance in breeding, morphological diversity and the genetic traits they represent.

DNA extraction: Plant DNA will be extracted from the young leaves (5g) using the modified CTAB method (Doyle and Doyle 1987).

***Eco* RI sub-genomic library construction:** Total plant DNA isolated from the etiolated seedlings of chickpea accession ICC 4918 will be digested with restriction enzyme *Eco*RI and size fractionated in 0.8% low melting agarose gel. DNA fragments of 2kb to 3kb size will be eluted from the gel and ligated to plasmid vector pUC18. *Escherichia coli* (DH5α) bacterial cells will be then transformed with the ligated plasmid DNA. Colonies of cells containing the plasmid with chickpea DNA insert will be selected on the basis of X-gal/IPTG screening procedure (Davis et al. 1986). Individual colonies will be isolated and plasmid alkaline mini pre-prepared (Birnboim and Doly 1979) and random genomic clones will be denoted with consecutive numbers (CGE #)

DNA probes: Genomic clones will be isolate from the *Pst* I sub-genomic library (CG clones) (Udupa *et al.* 1993); *Eco* RI sub-genomic library (CGE clones) and one heterologous nuclear DNA probe, *Cab*3C, from tomato (gift from E.Pichersky, Univ. Of Michigan, USA) will be used as probes for RFLP analysis.

Restriction digest, electrophoresis and Southern analysis: Five microgram of DNA from each individual accession was digested with restriction enzymes *Eco* RV, *Xba* I, *Hind* III, *Msp* I, *Hpa* II, *HInf* I, *Dra* I and *Pvu* II (*Stratagene*, La Jolla, USA and *Amersham*, Buckinghamshire, UK) at conditions specified for each enzyme. Electrophoresis of the digested plant DNA, Southern analysis will be done as per the Udupa *et al*. (1993).

Primers and RAPD analysis: Random decamer primers (*OPERON Tech. Inc.,* Alameda, USA) will be tested. Forty primers OPA (A-4, 7, 8, 9, 11, 13, 14), OPB (B-1, 4, 5, 19, 20), OPC (C-4, 19), OPD (D-1, 2, 3, 5, 7, 8, 11, 13, 15), OPF (F-1, 3, 4, 6, 7, 9, 11, 16) and OPI (I-1, 3, 4, 5, 6, 7, 13, 16, 20) will be used for RAPD analysis. PCR amplification will be performed according to Williams et al. (1990). Amplification reaction will be performed in 0.2 cm^3 thin-walled PCR tubes (*Micro-Amp* reaction tubes, *Perkin Elmer*, Norwalk, USA) containing 1 X reaction buffer [10 mM Tris- HCL (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$ and 0.01% gelatine (m/v)], 120 µM of each dNTP from a 10 mM stock (*Perkin Elmer*, Norwalk, USA), 15ng of primer, 25ng of the template DNA and 2 units of *Taq* DNA polymerase (*Stratagene*, La Jolla, USA). Amplification will be carried out in a thermal- cycler. The PCR products will be electrophoresed in a 1.2 % agarose gel in 1X TAE buffer (Tris-acetate 0.04 M, EDTA 0.001 M, Ph 7.8) at 40 V for 5 h, stained with ethidium bromide and will be visualised by illumination against UV light and recorded on *Polaroid* type 667 film (*Polaroid*, Cambridge, USA).

DNA Barcoding The selected chickpea species collected different location of country will be tested to detect genetic polymorphism using DNA barcoding. The evolution of genetically related group of organisms will be estimated using phylogenetic relationships obtained by MEGA 5.2 software. The phylogenetic analysis of selected species will be carried out using four methods *viz.*, minimum evolution, neighbor joining, UPGMA and maximum parsimony.

Data analysis: Each fragment of DNA produced during PCR amplification across all the accessions will be assigned a number (1,2, 3,) in order of decreasing base pair size. Each fragment will be treated as a unit character, and scored as 1 (presence) or 0 (absence) for each sample. Data will be analysed by both phonetic (cluster and principal co-ordinate analysis) and cladistic (neighbour-joining method) approaches. Phenogram based on similarities will be constructed via the UPGMA using NTSYS- pc 1.60 microcomputer program (Rohlf 1993). Dendrograms based on neighbour-joining (NBJ) method will be constructed (Saitou and Nei 1987).

3. Statistical analysis:

Statics is the discipline that concern the collection, organization, analysis, interpretation and presentation of data. In applying statistics to a scientific industrial or social problem.

5. Importance of study/ society application:

Chick pea is a one of the major commercial crops in India. So how we increase the production of chick pea is a major problem in India. Different region of India has different productivity rate per hector of land. the study reveals that for improving seed yield and developing high yielding genotype of chick pea due to emphasis should be placed on number of fruiting branches per plant, number of pods per plant and days to maturity. as all these traits has high heritability coupled with high to moderate genetic advance, it may be possible to have a rapid gain by mass selection. since, late flowering and maturity beyond certain limit is not desirable, greater emphasis should be laid on early flowering and early maturity genotype. Simply this study reveals that how to increase production per hector of land, how to manage different diseases, how genetic makeup of different chick pea seed impact on crop production and how different region affect crop production.

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Research Student **Research Guide**

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